

### **AMENDMENTS TO THE CLAIMS:**

Please cancel claims 1-32 and add the new claims as follows:

Claim 33. (New) Use of inhibitors of h-Prune cyclic nucleotide phosphodiesterase activity for the preparation of a medicament for prevention and treatment of tumour metastases characterised by an overexpression of h-PRUNE, said inhibitors being selected from the group consisting of a peptide having the following amino acidic sequence NIIHGSDSVESAEKE (SEQ ID No 9); a peptide comprising the following amino acidic sequence NIIHGSDSVESAEKE GGGYGRKKRRQRRR (SEQ ID No 10); vinpocetine, IC261 and derivatives, structural analogues and isomers thereof.

Claim 34. (New) Use according to claim 33, wherein tumours characterised by an overexpression of h-PRUNE are breast carcinoma, sarcoma, neuroblastoma, prostate tumour, pancreatic tumour, colon carcinoma tumour, rectal tumour, medulloblastoma, epithelioma, epatocarcinoma, cell T or cell B lymphomas, myeloma and melanoma, and pulmonary tumour.

Claim 35. (New) Peptide comprising the following amino acidic sequence: NIIHGSDSVESAEKEGGGYGRKKRRQRRR (SEQ ID No 10) characterised in that it is permeable.

Claim 36. (New) Peptide comprising the following amino acidic sequence: NIIHGSDSVESAEKE GGGYGRKKRRQRRR (SEQ ID No 10) and characterised in that it is permeable and it is an inhibitor of h-Prune cyclic nucleotide phosphodiesterase activity, for use in medical field.

Claim 37. (New) Peptide having the following amino acidic sequence: NIIHGSDSVESAEKE (SEQ ID No 9).

Claim 38. (New) Peptide having the following amino acidic sequence: NIIHGSDSVESAEKE (SEQ ID No 9) characterised in that it is an inhibitor of h-Prune cyclic nucleotide phosphodiesterase activity, for use in medical field.

Claim 39. (New) Screening method for h-PRUNE-inhibiting compounds, comprising the following phases:

- a) selection of at least a phosphoesterase (PDE) inhibiting compound or derivative, structural analogue or isomer thereof;
- b) administration of said at least one compound at concentration between 0,05  $\mu\text{M}$  and 10  $\mu\text{M}$  in a cell line overexpressing h-PRUNE, wherein said cellular line is MDA-C100 435 prune #4;
- c) quantitative analysis of the cyclic nucleotide phosphodiesterase activity of h-PRUNE and/or analysis of cellular motility versus concentration of said at least one compound and chemo-attractant and selection of compound able to inhibit said phosphodiesterase activity between the values from 0.01 to 1  $\text{pmol}/\text{min}^{-1}/\text{ug}^{-1}$  and/or inhibit said motility up to the attainment of the values between 200 and 1200 cells.

Claim 40. (New) Screening method according to claim 39, wherein the quantitative analysis of step c) is carried out by hydrolysis tests of the c-AMP and/or c-GMP substrate.

Claim 41. (New) Screening method according to claim 39, wherein the substrate is used at concentration between 0,008  $\mu\text{M}$  and 1  $\mu\text{M}$ .

Claim 42. (New) Method for in vitro detection of h-PRUNE in a biological sample for metastases diagnosis of tumours characterised by an h-PRUNE overexpression by immunological assay, FISH analysis, Real-time PCR, in situ hybridization.

Claim 43. (New) Method for in vitro detection of h-PRUNE according to claim 42, comprising the following steps:

- a) bring into contact said biological sample with at least one anti-h-PRUNE monoclonal antibody;
- b) detection of the antigen-antibody complex;
- c) quantitative analysis of the antigen-antibody complex.

Claim 44. (New) Method according to claim 43, Wherein said biological sample is a tissue section or biological fluid.

Claim 45. (New) Method according to claim 42, wherein said anti-h-PRUNE antibody is the monoclonal antibody able to recognise and bind selectively the h-PRUNE recombinant protein, characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).

Claim 46. (New) Method according to claim 42, wherein said anti-h-PRUNE antibody is labelled with a radioisotope, fluorescent molecule or enzyme.

Claim 47. (New) Method for in vitro detection of h-PRUNE according to claim 43, wherein said detection and quantitative analysis of the antigenantibody complex are performed by immunohistochemistry, immunoprecipitation, immunofluorescence, ELISA, immunoblotting analyses.

Claim 48. (New) Method according to claim 42, wherein PCR Real time primers specific for h-PRUNE comprise the sequences:

5'-AGAGATCTTGGACAGGCAAAC-3' (SEQ ID No 1);

3'-CCATGTTGACACAGTCCAGGAT-5' (SEQ ID No 2);

or their complementary sequences.

Claim 49. (New) Method according to claim 42, wherein the labelled probe for Real-time PCR or in situ hybridization comprise the oligonucleotidic sequence: CTGCATGGAACCATC (SEQ ID No 3) or its complementary sequence or the sequence wherein T is replaced by U.

Claim 50. (New) Method according to claim 49, wherein said labelled probe for Real-time PCR is linear or circular one.

Claim 51. (New) Method according to claim 49, wherein said probe is labelled with at least one radioisotope and/or fluorochrome.

Claim 52. (New) Method according to claim 49, wherein said probe is labelled with at least a fluorochrome at 5' and/or 3'.

Claim 53. (New) Method according to claim 49, wherein said fluorochrome is 6-carboxifluorescein.

Claim 54. (New) Diagnostic kit for the detection of h-PRUNE in a biological sample for metastases diagnosis of tumours characterised by an h-PRUNE overexpression comprising at least one anti-h-PRUNE monoclonal antibody, or a pair of primers specific for h-PRUNE or labelled oligonucleotidic probe specific for h-PRUNE.

Claim 55. (New) Diagnostic kit according to claim 54, wherein the tumours characterised by an h-PRUNE overexpression are breast carcinoma, sarcoma, neuroblastoma, melanoma.

Claim 56. (New) Diagnostic kit according to claim 54, wherein said anti-h-PRUNE antibody is characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).

Claim 57. (New) Diagnostic kit according to claim 56, wherein said anti-h-PRUNE monoclonal antibody is labelled with a radioisotope, fluorescent molecule or enzyme.

Claim 58. (New) Diagnostic kit according to claim 54, wherein said pair of primers specific for h-PRUNE comprises the sequences:

5'-AGAGATCTTGGACAGGCAAAC-3' (SEQ ID No 1);

3'-CCATGTTGACACAGTCCAGGAT-5' (SEQ ID No 2);

or their complementary sequences.

Claim 59. (New). Diagnostic kit according to claim 54, wherein said labelled oligonucleotidic probe for Real-time PCR or in situ hybridization comprises the oligonucleotidic sequence:

CTGCATGGAACCATC (SEQ ID NO 3)

or its complementary sequence or the sequence wherein T is replaced by U.

Claim 60. (New) Diagnostic kit according to claim 59, wherein said labelled oligonucleotidic probe for Real-time PCR is linear or circular one.

Claim 61. (New) Diagnostic kit according to claim 59, wherein said oligonucleotidic probe is labelled with at least one radioisotope and/or fluorochrome.

Claim 62. (New) Diagnostic kit according to claim 59, wherein said probe is labelled with at least one fluorochrome at 5' and/or 3'.

Claim 63. (New) Diagnostic kit according to claim 62, wherein the fluorochrome is 6-carboxyfluorescein.

Claim 64. (New) Monoclonal murine antibody able to recognise and bind selectively the h-PRUNE recombinant protein, characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).